Overexpression of human β2-adrenergic receptors increases gain of excitation-contraction coupling in mouse ventricular myocytes

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Grandy, Scott A., Eileen M. Denovan-Wright, Gregory R. Ferrier, and Susan E. Howlett. Overexpression of human β2-adrenergic receptors increases gain of excitation-contraction coupling in mouse ventricular myocytes. Am J Physiol Heart Circ Physiol 287: H1029–H1038, 2004. First published May 20, 2004; 10.1152/ajpheart.00814.2003.—This study investigated cardiac excitation-contraction coupling at 37°C in transgenic mice with cardiac-specific overexpression of human β2-adrenergic receptors (TG4 mice). In field-stimulated myocytes, contraction was significantly greater in TG4 compared with wild-type (WT) ventricular myocytes. In contrast, when duration of depolarization was controlled with rectangular voltage clamp steps, contraction amplitudes initiated by test steps were the same in WT and TG4 myocytes. When cells were voltage clamped with action potentials simulating TG4 and WT action potentials, contractions were greater with long TG4 action potentials and shorter with shorter WT action potentials, which suggests an important role for action potential configuration. Interestingly, peak amplitude of L-type Ca2+ current (I_{Ca-L}) initiated by rectangular test steps was reduced, although the voltage dependencies of contractions and currents were not altered. To explore the basis for this, we measured Ca2+ transients in myocytes loaded with fura 2. Diastolic concentrations of free Ca2+ and amplitudes of Ca2+ transients were similar in voltage-clamped myocytes from WT and TG4 mice. However, sarcoplasmic reticulum (SR) Ca2+ content assessed with the rapid application of caffeine was elevated in TG4 cells. Increased SR Ca2+ was accompanied by increased frequency and amplitudes of spontaneous Ca2+ sparks measured at 37°C with fluo 3. These observations suggest that the gain of Ca2+-induced Ca2+ release is increased in TG4 myocytes. Increased gain counteracts the effects of decreased amplitude of I_{Ca-L} in voltage-clamped myocytes and likely contributes to increased contraction amplitudes in field-stimulated TG4 myocytes.

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To evaluate the effects of β2-AR overexpression on cardiac EC coupling it is essential to measure differences in contraction, Ca\textsuperscript{2+} transients, I\textsubscript{Ca-L}, and SR Ca\textsuperscript{2+} content either simultaneously or under comparable experimental conditions. The objectives of this study were 1) to compare amplitudes of contraction in field-stimulated TG4 and WT ventricular myocytes at physiological temperature, 2) to measure contraction and I\textsubscript{Ca-L} simultaneously in voltage-clamped ventricular myocytes where effects of action potential configuration are eliminated, 3) to assess SR Ca\textsuperscript{2+} content and Ca\textsuperscript{2+} transients with fura 2 under conditions identical to those used to evaluate contraction and I\textsubscript{Ca-L}, and 4) to compare the frequency and properties of Ca\textsuperscript{2+} sparks at physiological temperature in TG4 and WT mouse myocytes.

METHODS

Cell isolation. Experiments were conducted on cardiac ventricular myocytes from 24- to 32-wk-old male and female TG4 mice and WT littermates. Initial breeding pairs were made up of female WT (B6SJLF1/J) and male TG4 [B6SJL-TgN(Wtbeta2)4Wjk] mice obtained from Jackson Laboratories. The colony was then maintained by breeding TG4 animals with WT littermates. Because TG4 mice are (B6SJLF1/J) and male TG4 [B6SJL-TgN(Wtbeta2)4Wjk] mice obtained from Jackson Laboratories. The colony was then maintained by breeding TG4 animals with WT littermates. Because TG4 mice are (B6SJLF1/J) and male TG4 mice ob-

contraction in simultaneously or under comparable experimental conditions. The transients were elicited by voltage clamping from −80 to 0 mV. Fura 2 Ca\textsuperscript{2+} transients elicited by rapid application of caffeine were used as an index of SR Ca\textsuperscript{2+} load in voltage-clamped cells (2, 24). After five 200-ms conditioning pulses to 0 mV, cells were repolarized to −60 mV and caffeine was applied with a rapid solution switcher for 1 s. The switcher is a computer-controlled device that allows complete change of the solution bathing the myocyte in <0.5 s while maintaining the temperature at 37°C (15). Caffeine was applied in a solution of the following composition (in mM): 10 caffeine, 140 LiCl, 4 KCl, 10 glucose, 5 HEPES, 4 MgCl\textsubscript{2}, 2.5 4-aminopyridine, and 2.5 lidocaine. Na\textsuperscript{+} and Ca\textsuperscript{2+} were omitted from this solution to minimize loss of Ca\textsuperscript{2+} through Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange (17). However, because Na\textsuperscript{+} and Ca\textsuperscript{2+}-free solution was not applied in advance of caffeine it is possible that some Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange could occur during the initial period of caffeine application.

Ca\textsuperscript{2+} sparks. Ca\textsuperscript{2+} sparks were recorded with a Zeiss LSM 510 laser scanning confocal microscope with techniques described previously (8). Myocytes were incubated with 20 μM fluo 3-AM for 25 min. Cells were transferred to an experimental chamber on the microscope stage and superfused with the standard buffer solution at 37°C. Ca\textsuperscript{2+} sparks were measured in quiescent WT and TG4 myocytes. Changes in free Ca\textsuperscript{2+} were detected in line scan mode with excitation at 488 nm and emission measured at 525 nm (Zeiss oil-immersion objective, ×40, 1.3 numerical aperture). Cells were repetitively scanned along the length of the cell at 1.5-ms intervals, for a maximum of 6 s. Each line was composed of 512 pixels. The confocal pinhole was adjusted to maximize x-y-z resolution to 0.26 × 0.26 × 0.75 μm. Laser intensity was reduced to ≤5% maximum to minimize cytotoxicity and dye bleaching. Line scan diagrams were constructed by stacking emission lines in temporal order.

Data analysis. Contraction amplitude was measured as peak shortening with respect to cell length immediately before cell shortening. Contraction measurements were normalized as percent resting cell length, to allow comparison of cell shortening between the TG4 and WT groups. Time to peak contraction was measured as time between the initiation of contraction and maximal cell shortening, and time to half-relaxation was the time required for contraction to relax by 50%. Depolarization- and caffeine-induced Ca\textsuperscript{2+} transients were measured with reference to diastolic [Ca\textsuperscript{2+}] (Fig. 1A). We used the Ca\textsuperscript{2+} channel blocker Cd\textsuperscript{2+} to validate our measurements of I\textsubscript{Ca-L}. In these experiments, we used the rapid solution switcher to apply Cd\textsuperscript{2+} for 3 s after the conditioning pulse train and during the test step (Fig. 1). We subtracted the current in the presence of Cd\textsuperscript{2+} (Fig. 1B) from the current in the absence of Cd\textsuperscript{2+} (Fig. 1A) to obtain the Cd\textsuperscript{2+}-sensitive current (Fig. 1C). We then compared the amplitudes of peak Cd\textsuperscript{2+}-sensitive current with amplitudes of peak I\textsubscript{Ca-L} measured as the difference between peak inward current and a reference point at the end of the voltage step. There was no significant difference in amplitudes of I\textsubscript{Ca-L} measured with the two techniques. Therefore, we measured the amplitude of I\textsubscript{Ca-L} as the difference between peak inward current and a reference point at the end of the voltage step. Cell membrane area was determined by integrating capacitive transients with pCLAMP software; I\textsubscript{Ca-L} was normalized by cell capacitance and expressed as current density.

Ca\textsuperscript{2+} sparks were visualized and analyzed with Image J (National Institutes of Health). Ca\textsuperscript{2+} sparks were identified as local peak elevations of fluorescent intensity (F) that were ≥1.5 times the surrounding background levels (F\textsubscript{0}). Both the percentage of cells...
RESULTS

The first series of experiments compared the amplitudes of contraction of WT and TG4 myocytes at 37°C in cells that were field stimulated at 2 Hz. Representative recordings of unloaded cell shortening from WT and TG4 myocytes are shown in Fig. 2, A and B, respectively. The amplitude of contraction was much greater in the TG4 myocyte than in the WT cell, although the time courses of contractions were similar. Mean data for these parameters are shown in Fig. 2, C–F. Figure 2C shows that the amplitudes of contraction were significantly greater in TG4 myocytes compared with WT controls. However, diastolic cell length (Fig. 2D) was similar in the two groups, as were time to peak shortening (Fig. 2E) and time to half-relaxation (Fig. 2F). Thus, although the amplitude of contraction was significantly greater in field-stimulated TG4 cells at 37°C, neither time course of contrac-

eating Ca²⁺ sparks (incidence) and frequency of Ca²⁺ sparks (sparks·100 μm⁻¹·s⁻¹) were calculated for WT and TG4 myocytes. Amplitude (F/F₀), full spatial width at half-maximum amplitude (FWHM), time to peak amplitude, and time to half-decay were calculated for individual sparks to characterize and compare sparks in WT and TG4 myocytes.

Statistical analyses. The differences between means for WT and TG4 groups for the field stimulation data, voltage clamp data, fluorescence data and spark data were assessed with a t-test. Differences in contraction-voltage and current-voltage relationships between the two groups were evaluated with a two-way repeated-measures ANOVA. Differences in incidence of Ca²⁺ sparks between WT and TG4 cells were assessed with a χ²-test. t-Test and ANOVA calculations were performed with SigmaStat 2.03 (Jandel) or SAS (SAS Canada, Toronto, ON, Canada). Data are presented as means ± SE. No more than two myocytes from one heart were included in any one data set.

Chemicals. Lidocaine, HEPES buffer, EGTA, MgCl₂, anhydrous DMSO, 4-aminopyridine, and caffeine were purchased from Sigma-Aldrich Canada (Oakville, ON, Canada). Fura 2-AM, fluo 3-AM, and Pluronic F-127 were purchased from Molecular Probes (Hornby, ON, Canada). All other chemicals were purchased from BDH (Toronto, ON, Canada). Fluo 3-AM, and fluo 3-AM, and Pluronic F-127 in 12 ml of fetal calf serum (Invitrogen, Burlington, ON, Canada). All other chemicals were dissolved in deionized water.

Fig. 1. Measurements of L-type Ca²⁺ current (I_{Ca,L}) reflect the Cd²⁺-sensitive current in mouse ventricular myocytes. Cells were voltage clamped with the protocol shown at top. A: a voltage clamp step from −65 to −40 mV was used to discharge and inactivate Na⁺ current. A second step from −40 to 0 mV activated inward current. B: inward current was abolished by rapid application of 100 μM Cd²⁺. C: Cd²⁺-sensitive difference current was obtained by subtraction of the current in the presence of Cd²⁺ from the current in the absence of Cd²⁺. D: amplitude of I_{Ca,L} measured as the peak Cd²⁺-sensitive current is similar to the amplitude of peak I_{Ca,L} measured as the difference between peak inward current and a reference point at the end of the voltage step, n = 12 Wild-type (WT) myocytes.

Fig. 2. Amplitudes of contraction were significantly greater in field-stimulated TG4 myocytes compared with WT controls. WT and TG4 myocytes were field stimulated at 2 Hz in experiments at 37°C. A and B: representative contractions for WT and TG4 myocytes, respectively. C: mean WT and TG4 contraction data show that amplitudes of contraction were significantly larger in TG4 myocytes compared with WT cells. Cell shortening is expressed as % of maximum cell length. D: diastolic cell length was similar in WT and TG4 cells. There was no significant difference in time to peak contraction (E) or time to half-relaxation (F) between the two groups. *Significantly different from WT (P < 0.05) (n = 5–9 WT myocytes and 5 TG4 myocytes).
tion nor diastolic cell length was different between the two groups.

We next determined whether the difference in amplitudes of contraction between WT and TG4 cells persisted when the duration of depolarization was controlled by voltage clamp. Test steps were preceded by trains of five 200-ms-long conditioning pulses (2 Hz, −80 to 0 mV) to provide comparable activation histories in each cell (Fig. 3, top). Trains of conditioning pulses were followed by a postconditioning potential of either −40 or −65 mV. Test steps to activate transmembrane currents and contractions were made from the postconditioning potential. Figure 3, A and B, shows representative recordings of contractions and currents from WT and TG4 cells depolarized with a 250-ms test step from −65 to 0 mV. Interestingly, when cells were voltage clamped, there was no difference in the amplitudes of contraction between WT and TG4 myocytes. Furthermore, $I_{Ca,L}$ was smaller in the TG4 myocyte compared with the WT cell (Fig. 3, A and B). Mean data for these experiments are shown in Fig. 3C. Figure 3C, top, shows that the amplitudes of contraction were not significantly different between WT and TG4 myocytes. However, the magnitude of peak $I_{Ca,L}$ was significantly smaller in TG4 myocytes than in WT cells (Fig. 3C, bottom).

We showed previously (9, 16) that components of both contraction and inward current may be inactivated when a postconditioning potential of −40 mV is used. Therefore, in an additional series of experiments cells were depolarized from −65 mV. Mean data for these experiments are shown in Fig. 3D. Comparison of Fig. 3, C and D, shows that contractions were larger in magnitude when cells were activated from −65 mV, and this increase was significant for the WT cells. However, there still was no significant difference between the amplitudes of contractions recorded from WT and TG4 myocytes. In addition, peak inward current remained significantly smaller in TG4 myocytes despite the change in postconditioning potential. We also determined whether the time course of contraction activated by voltage clamp steps differed between WT and TG4 myocytes. Mean data presented in Fig. 3E show that both times to peak contraction and half-relaxation times were similar in WT and TG4 myocytes when cells were activated by steps from −40 mV. Similar results were obtained when cells were activated by steps from −65 mV (Fig. 3F).

Our results demonstrate that contractions are larger in field-stimulated TG4 cells than in WT myocytes, although these differences are abolished when the cells are depolarized with a rectangular voltage-clamp test step. Because action potential duration is prolonged in TG4 myocytes compared with WT cells (33), it is possible that differences in contraction amplitudes between TG4 and WT myocytes may reflect differences in action potential duration. To test this possibility we used simulated action potential waveforms and conducted experiments under voltage-clamp conditions. We used previously published values for resting potentials, action potential amplitudes, and action potential durations at 50% and 90% repolarization to simulate action potentials from WT and TG4 mouse myocytes (33). All values were similar in WT and TG4 cells except that action potential duration at 90% repolarization was 48 ms in WT cells and 104 ms in TG4 cells (33). Cells were held at the resting potential of −70 mV and stimulated with trains of either WT or TG4 action potentials, and contractions were recorded. Figure 4A shows a representative simulated WT action potential (top) and contraction (bottom) recorded from a WT cell. Figure 4B shows that contraction increased markedly when the same cell was activated with a simulated TG4 action potential. Mean data demonstrate that the increase in action potential duration caused a significant increase in magnitudes of contractions (Fig. 4C). Thus prolongation of the action potential could contribute to the increase in magnitude of contraction in field-stimulated TG4 myocytes.

The smaller amplitude of peak $I_{Ca,L}$ in TG4 myocytes could be caused either by a reduction in maximum current or by a shift in the current-voltage relationship. To differentiate between these possibilities the voltage dependencies of contraction and current were determined. The voltage-clamp protocol is illustrated in Fig. 5, top. In these experiments, test steps were initiated from a postconditioning potential of −40 mV after trains of conditioning pulses. Test steps to different potentials were made with each repetition of the protocol. Contraction and current amplitudes were plotted as a function of test step voltage to construct contraction-voltage and current-voltage relationships for WT and TG4 myocytes (Fig. 5, A and B). Figure 5A shows that the contraction-voltage relationships were bell-shaped with a peak near 0 mV. Similarly, current-voltage relationships were bell-shaped with peaks near 0 mV (Fig. 5B). Clearly, there were no shifts in the voltage dependence of either contraction or current in TG4 compared with WT myocytes. Interestingly, although contraction amplitudes were virtually identical in the two cell types, the amplitudes of $I_{Ca,L}$ were significantly smaller near the peak of the current-voltage relation in TG4 myocytes (Fig. 5B). Thus the amplitudes of contraction were maintained despite a significant reduction in the amplitude of $I_{Ca,L}$ in TG4 myocytes. These findings indicate that the relationship between $I_{Ca,L}$ and contraction is altered in TG4 myocytes and suggest that the gain of EC coupling is increased in TG4 myocytes.

It is not clear how full-sized contractions are maintained when peak inward $I_{Ca,L}$ is reduced −50% in TG4 myocytes. An increase in EC coupling gain in TG4 myocytes could reflect a change in myofilament Ca$^{2+}$ sensitivity and/or a change in the sensitivity of CICR. To explore the mechanism of altered EC coupling gain, we conducted a separate series of experiments to compare and characterize diastolic Ca$^{2+}$ concentrations and Ca$^{2+}$ transient amplitudes in TG4 and WT myocytes. In these experiments [Ca$^{2+}$], was measured with fura 2. Cells were held at a potential of −80 mV and depolarized with trains of voltage-clamp steps to 0 mV at a frequency of 2 Hz. Figure 6, A and B, shows representative Ca$^{2+}$ transients for WT and TG4 myocytes. The representative traces show that resting and peak Ca$^{2+}$ concentrations were similar in the two types of myocytes. Figure 6C shows that mean diastolic Ca$^{2+}$ concentrations were not significantly different between WT and TG4 myocytes. In addition, the mean amplitudes of Ca$^{2+}$ transients elicited by depolarizing steps also were not significantly different between cell types (Fig. 6D). These observations indicate that Ca$^{2+}$ transients, like contractions, have similar amplitudes in voltage-clamped TG4 and WT myocytes. Thus the difference in gain between cell types is not likely explained by a difference in myofilament Ca$^{2+}$ sensitivity. Rather, these data indicate that the higher gain is largely caused by release of a similar amount of SR Ca$^{2+}$ despite a smaller trigger influx of Ca$^{2+}$.
Fig. 3. The difference in amplitudes of contraction between WT and TG4 myocytes was abolished when cells were voltage clamped. After a train of conditioning pulses, cells were depolarized from either −65 or −40 mV to 0 mV as illustrated in the schematic at top. A and B: representative contractions (top) and currents (bottom) recorded from −65 mV in WT and TG4 cells, respectively. C: when cells were depolarized from −40 mV, mean contraction amplitudes (top) were similar in WT and TG4 myocytes. In contrast, mean peak \( I_{Ca,L} \) (bottom) was significantly smaller in TG4 myocytes than WT. D: similar results were obtained when cells were depolarized from −65 mV. E: when cells were depolarized from −40 mV, mean times to peak contraction (top) and half-relaxation times (bottom) were similar in WT and TG4 myocytes. F: mean time courses of contraction also were similar in WT and TG4 myocytes when cells were depolarized from −65 mV. *Significantly different from WT \( (P < 0.05) \); †significantly different from a postconditioning potential of −40 mV \( (P < 0.05) \) \((n = 12–13 \text{ WT myocytes and 6–8 TG4 myocytes})\).
Several studies have shown that the sensitivity of CICR is increased when SR Ca$^{2+}$ load is elevated (1, 20, 28). Therefore, we explored the possibility that increased gain in TG4 myocytes is accompanied by increased SR Ca$^{2+}$ stores relative to WT myocytes. These experiments were conducted in voltage-clamped cells loaded with fura 2. SR Ca$^{2+}$ content was assessed by rapid application of 10 mM caffeine for 1 s. The voltage-clamp protocol is shown in Fig. 7 (inset). Cells were activated with five conditioning pulses delivered at a frequency of 2 Hz. Cells were then repolarized to −60 mV, and caffeine was applied with a rapid solution switcher. Caffeine was applied in a 0 mM Na$^{+}$-0 mM Ca$^{2+}$ solution to minimize loss of Ca$^{2+}$ through the Na$^{+}$/Ca$^{2+}$ exchanger (17). Figure 7, A and B, shows representative caffeine-induced Ca$^{2+}$ transients recorded from WT and TG4 myocytes. The caffeine-induced Ca$^{2+}$ transient was larger in the TG4 myocyte than in the WT myocyte. Mean data for these experiments are presented in Fig. 7C. Mean amplitudes of caffeine-induced Ca$^{2+}$ transients were significantly greater in TG4 myocytes than in WT cells. These results show that SR Ca$^{2+}$ stores are significantly greater in TG4 myocytes than in WT myocytes and therefore may be responsible for the increased gain of EC coupling observed in TG4 cells.

To determine whether Ca$^{2+}$ release was sensitized at the level of the fundamental SR Ca$^{2+}$ release units, we measured and compared the occurrence and characteristics of spontaneous Ca$^{2+}$ sparks in WT and TG4 cells. These experiments were conducted in non-voltage-clamped myocytes. Sparks were detected at physiological temperature because temperature is known to alter the frequency and characteristics of Ca$^{2+}$ sparks (8). Figure 8, A and B, shows representative line scan diagrams, which illustrate spontaneous Ca$^{2+}$ sparks recorded from WT and TG4 myocytes. More Ca$^{2+}$ sparks are apparent in the TG4 line scan. Furthermore, the sparks recorded from the TG4 cell appeared brighter than sparks recorded from the WT cell. The fraction of cells exhibiting spontaneous sparks within line scans with durations of 6 s also was markedly different between cell types. This is illustrated in Fig. 8C, which shows that >90% of TG4 cells exhibited spontaneous Ca$^{2+}$ sparks whereas only 20% of WT cells exhibited spontaneous sparks. The difference in incidence of cells exhibiting sparks was significant. The frequency of Ca$^{2+}$ sparks also was significantly greater in TG4 myocytes than in WT myocytes and therefore may be responsible for the increased gain of EC coupling observed in TG4 myocytes compared with WT controls, as shown in Fig. 8D. Thus Ca$^{2+}$ release events were increased in both incidence and frequency in TG4 myocytes compared with WT cells.

Fig. 4. Contraction amplitudes are greater when cells are voltage clamped with action potentials designed to mimic TG4 action potentials than when simulated WT action potentials are used. A: a representative WT mouse ventricular myocyte was voltage clamped with a simulated action potential waveform designed to mimic a WT action potential (top). The simulated WT action potential triggered a contraction (bottom). B: duration (time to 90% repolarization) of the simulated action potential in A was increased from 48 ms to 104 ms to mimic a TG4 action potential (top). The amplitude of contraction of the same myocyte shown in A increased when action potential duration was prolonged (bottom). C: mean amplitudes of contraction increased when myocytes were voltage clamped with simulated TG4 action potentials over those when cells were voltage clamped with simulated WT action potentials. *Significantly different from WT (P < 0.05) (n = 13 myocytes).

Fig. 5. Contraction amplitude is maintained in TG4 myocytes despite a significant reduction in the magnitude of $I_{Ca-L}$. Cells were depolarized to different potentials in 10-mV increments from −40 to +60 mV (top). A: mean contraction-voltage relationships were very similar in WT and TG4 cells. B: mean current-voltage relationships recorded from WT and TG4 cells showed that the maximum amplitude of $I_{Ca-L}$ was smaller in TG4 cells than in WT myocytes. This difference was statistically significant at membrane potentials from −10 to +20 mV. *Significantly different from WT (P < 0.05) (n = 12 WT myocytes and 6 TG4 myocytes).
The goal of this study was to evaluate the effects of β2-AR overexpression on cardiac EC coupling. In particular, we wished to explore the relationship between amplitudes of I_{Ca,L} overexpression on cardiac EC coupling. In particular, we measured the amplitudes, spatial widths, and time courses of individual spontaneous Ca^{2+} sparks in WT and TG4 cells at physiological temperature. Figure 9, A–D, compares mean values for these parameters in WT and TG4 myocytes. Figure 9A shows that the amplitude of Ca^{2+} sparks, expressed as F/F_0, was significantly greater in TG4 myocytes compared with WT cells. However, the spatial width, measured as FWHM, was not significantly different between the two groups (Fig. 9B). Similarly, neither the time to peak amplitude nor the time to half-decay of Ca^{2+} sparks differed significantly in WT and TG4 myocytes (Fig. 9, C and D). Thus Ca^{2+} sparks had greater amplitudes and occurred with a greater frequency in TG4 myocytes.

DISCUSSION

We also measured the amplitudes, spatial widths, and time courses of individual spontaneous Ca^{2+} sparks in WT and TG4 cells stimulated at 2 Hz in the presence of 1 mM Ca^{2+}. We found that the amplitude of contraction was larger in field-stimulated TG4 cells compared with WT myocytes, although there was little difference in time to peak contraction and time to half-relaxation. Earlier studies reported that contractions are either larger (29, 31–33) or unchanged (11, 14) in field-stimulated TG4 myocytes. These contractions were accompanied by sensitization of Ca^{2+} release from SR Ca^{2+} stores, which is characteristic of TG4 cells. When cells were voltage clamped with action potentials designed to mimic TG4 action potentials, contraction amplitudes were greater than when simulated WT action potentials were used. In experiments with rectangular voltage clamp steps, simultaneous measurement of Ca^{2+} current in the same cells showed that peak I_{Ca,L} was markedly smaller in the TG4 myocytes. In addition, the amplitudes of Ca^{2+} sparks did not differ between the two groups of cells. Because the amplitudes of contractions and Ca^{2+} sparks were maintained despite a reduction in the amplitude of peak I_{Ca,L}, these results suggest that the gain of CICR is increased in TG4 myocytes. This increase in gain might reflect SR Ca^{2+} load, which was significantly greater in TG4 myocytes. Increased SR Ca^{2+} load was accompanied by sensitization of Ca^{2+} release as indicated by increased incidence, frequency, and amplitude of spontaneous Ca^{2+} sparks compared with WT myocytes.

In the present study, we examined EC coupling at 37°C in myocytes from 24- to 32-wk-old mice stimulated at 2 Hz in the presence of 1 mM Ca^{2+}. We found that the amplitude of contraction was larger in field-stimulated TG4 cells compared with WT myocytes, although there was little difference in time to peak contraction and time to half-relaxation. Earlier studies reported that contractions are either larger (29, 31–33) or unchanged (11, 14) in field-stimulated TG4 myocytes. These
studies were conducted at 22°C and 32°C, respectively, and utilized cells from animals of different ages, different stimulation rates, and different concentrations of extracellular Ca\(^{2+}\). Therefore, differences in results may reflect experimental conditions used in these studies. It also is possible that differences in action potential configuration may have contributed to the conflicting results obtained with different experimental conditions in earlier studies. This is suggested by our observation that the difference in amplitudes of contraction between cell types disappeared when cells were voltage clamped to control the time course of depolarization.

We also found that the magnitude of \(I_{Ca-L}\) was significantly smaller in TG4 myocytes than in WT myocytes at 37°C. This result is in agreement with several studies conducted at room temperature with myocytes from TG4 mice (13, 14, 19). However, several other reports have concluded that there was no difference in the magnitudes of \(I_{Ca-L}\) between WT and TG4 myocytes (32, 33). These latter studies were conducted in myocytes from relatively young mice (2–3 mo), and it has been suggested that reduction in \(I_{Ca-L}\) may develop with age in TG4 mice (13). Indeed, all of the studies reporting a decrease in \(I_{Ca-L}\) (including the present study) were conducted in mice aged between 3 and 8 mo (13, 14). There are several possible explanations for a reduction in the magnitude of \(I_{Ca-L}\) in cells from TG4 mice: 1) increased Ca\(^{2+}\)-induced inactivation of \(I_{Ca-L}\) related to elevated SR stores of Ca\(^{2+}\), 2) decreased open probability of L-type Ca\(^{2+}\) channels related to β2-AR overexpression, and 3) reduced expression of L-type Ca\(^{2+}\) channels in TG4 myocytes. The present study presents evidence that it is unlikely that \(I_{Ca-L}\) is reduced in amplitude in response to increased Ca\(^{2+}\) release from the SR, as the amplitudes of Ca\(^{2+}\) transients were not different in WT and TG4 myocytes. On the other hand, L-type Ca\(^{2+}\) channels have been shown to have a reduced open probability in cell-attached patch-clamp studies in WT and TG4 myocytes (13). The decreased open time resulted in a decreased maximum ensemble current in TG4 myocytes. The same study also demonstrated that the maximum peak \(I_{Ca-L}\) in TG4 myocytes could be restored when myocytes were pretreated with pertussis toxin to disrupt inhibitory G protein function (13). Because β2-ARs are known to couple to inhibitory G proteins, this suggests that \(I_{Ca-L}\) is decreased because of constitutive β2-AR activity in TG4 myocytes (13). Furthermore, restoration of \(I_{Ca-L}\) by pertussis toxin suggests that expression of L-type channels was not reduced in TG4 myocytes.

![Fig. 8. Incidence and frequency of spontaneous Ca\(^{2+}\) sparks are greater in myocytes from TG4 mice than WT mice. A and B: line scan diagrams showing representative Ca\(^{2+}\) sparks recorded from WT and TG4 myocytes. Systolic Ca\(^{2+}\) levels are indicated in orange, and localized increases in extracellular Ca\(^{2+}\) corresponding to Ca\(^{2+}\) sparks are indicated in yellow. Ca\(^{2+}\) sparks were more frequent in the TG4 myocyte. C: % of cells exhibiting spontaneous Ca\(^{2+}\) sparks (incidence) was significantly higher in TG4 cells compared with WT cells, as determined by χ²-test. D: frequency of Ca\(^{2+}\) sparks was increased significantly in TG4 myocytes compared with WT cells. *Significantly different from WT (\(P < 0.05\)) (\(n = 25\) WT and 12 TG4 cells).](http://ajpheart.physiology.org/faithful)
It is not clear which of the changes observed in TG4 myocytes is primarily responsible for the altered EC coupling observed in these cells. It is possible that increased SR Ca\(^{2+}\) stores result, at least in part, from constitutive β\(_2\)-AR activity, which could promote SR Ca\(^{2+}\) uptake in TG4 myocytes (23, 24). However, Ca\(^{2+}\) dynamics in cardiac cells involve the interplay between influx, efflux, storage, and release of Ca\(^{2+}\). A balance between these factors is achieved at steady state, and perturbation of one or more of these factors may alter the others (6, 7).

Interestingly, an increase in SR Ca\(^{2+}\) stores may compensate for the decreased magnitude of trigger Ca\(^{2+}\) entering the cell as \(I_{\text{Ca,L}}\). It has been demonstrated that increased SR Ca\(^{2+}\) content sensitizes Ca\(^{2+}\)-release channels and can increase the gain of CICR (1, 20, 28). Eventually the increase in gain of CICR results in Ca\(^{2+}\) transients that are the same amplitude as those seen in WT myocytes, even though the amplitude of \(I_{\text{Ca,L}}\) is smaller. Sensitization of Ca\(^{2+}\)-release channels also can be detected as an increase in frequency of Ca\(^{2+}\) sparks (28) and agrees with the observations in the present study. This balance between SR stores and Ca\(^{2+}\) release may therefore explain the maintenance of normal contractions and Ca\(^{2+}\) transients despite a reduction in \(I_{\text{Ca,L}}\) in voltage-clamped TG4 myocytes.

The above considerations by themselves cannot explain the increase in contraction amplitudes observed in field-stimulated myocytes. One possibility is that there is an increase in myofilament Ca\(^{2+}\) sensitivity in TG4 myocytes compared with WT cells. However, this does not seem likely because Ca\(^{2+}\) transients and contractions measured in voltage-clamp experiments were similar in WT and TG4 myocytes. It also is possible that the increase in contraction in field-stimulated TG4 cells is related to differences in action potential duration in WT and TG4 cells, as reported previously (33). Although action potential amplitudes and early repolarization were similar, the time to 90% repolarization was doubled to ~104 ms in TG4 myocytes (33). Indeed, our studies demonstrated that when cells were voltage clamped with action potentials designed to mimic TG4 action potentials, contractions were significantly larger than when simulated WT action potentials were used. It is possible that slowed repolarization in TG4 myocytes could increase Ca\(^{2+}\) influx by slowing deactivation of \(I_{\text{Ca,L}}\), even though peak \(I_{\text{Ca,L}}\) is reduced in TG4 myocytes. Slowed repolarization also may reduce efflux of released Ca\(^{2+}\) through the sarcolemma by reducing the electrochemical gradient for Ca\(^{2+}\) efflux through Na\(^{+}\)/Ca\(^{2+}\) exchange. Both of these effects would be expected to increase peak cytosolic Ca\(^{2+}\) levels and amplitudes of contraction in TG4 myocytes. Thus it is possible that the increased amplitude of contraction in field-stimulated myocytes with β\(_2\)-AR overexpression results from a further shift in the balance between Ca\(^{2+}\) influx and efflux related to prolongation of action potential duration.

The results of this study demonstrate that overexpression of nonnative β\(_2\)-ARs in murine cardiac myocytes can dramatically alter cardiac EC coupling at the cellular level. These findings are potentially important, because overexpression of β\(_2\)-ARs may have therapeutic potential in augmentation of contraction in diseases such as heart failure where β-AR signaling is disrupted (27). Therefore, it is important to understand the consequences of β\(_2\)-AR overexpression on cardiac function and EC coupling. However, the findings reported here apply only to overexpression of nonnative β\(_2\)-ARs in murine ventricular myocytes. Whether overexpression of human β\(_2\)-ARs receptors would produce similar effects on EC coupling in human ventricular myocytes is not clear and will require further investigation.

The present study supports earlier studies that reported that overexpression of β\(_2\)-ARs is accompanied by an increase in magnitude of contraction in field-stimulated myocytes and whole animals (19, 22, 31–33). However, the present study, conducted at physiological temperature, further investigates this observation by exploring the relationship between \(I_{\text{Ca,L}}\) and contraction measured simultaneously in the same cells. This study shows that the positive inotropic effect disappears in voltage-clamped myocytes and that the magnitude of \(I_{\text{Ca,L}}\) is actually smaller in TG4 myocytes than in WT myocytes. Nonetheless, the gain of CICR is clearly increased and likely is related to increased SR Ca\(^{2+}\) stores. Thus our results show that cardiac EC coupling is modified in myocytes overexpressing β\(_2\)-AR by changes that include reduced magnitude of \(I_{\text{Ca,L}}\) coupled to increased SR Ca\(^{2+}\) stores and increased gain of CICR. Increased gain likely counteracts the effects of decreased amplitude of \(I_{\text{Ca,L}}\) in voltage-clamped myocytes and may contribute to increased contraction amplitudes in field-stimulated TG4 myocytes.

![Graphs showing changes in Ca\(^{2+}\) sparks recorded from TG4 myocytes compared to WT myocytes.](image-url)


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